Electrochemical Labeling for Oligonucleotide Probe Hybridization

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With the progress of the whole genomic structure program, demand for gene analysis and gene diagnoses using nucleic acid hybridization techniques is increasing more than never. So there has been considerable interest in developing reliable labeling methods for detecting oligonucleotide probes hybridization. In contrast to the traditional method of labeling using radioisotopes, fluoresence, electrochemical labeling method offers the advantages of being sensitive and rapid and unhazardous. So electrochemical labeled oligonucleotide probes hybridization based on intercalation are to be made widely available.

In this work, a synthesized 20-mer single stranded oligonucleotide probe having a mercaptohexyl group at the 5' end was immobilized on a gold electrode by chemisorption, which has been characterized using X-ray photoelectron spectroscopy and diffuse reflection Infrared spectroscopy. The obtained diffuse reflection IR spectrum is shown in Figure 1. The 1687cm^{-1} absorption band is assigned to double-bond stretching vibrations of the DNA bases, the 1078cm^{-1} band results from symmetric stretching vibrations of the phosphates, the 1038cm^{-1} band is due to C-O stretching vibration of the ester linkage, and the 967cm^{-1} band is assigned to O-P-O antisymmetric stretching vibration of ribose-phosphate .

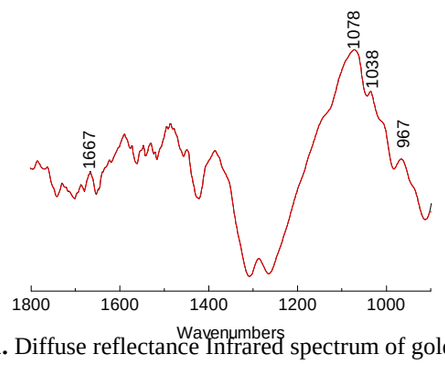

Fig.1. Diffuse reflectance Infrared spectrum of gold electrode modified with thiol-derivatized single stranded oligonucleotide

The hybridization reaction was detected using cyclic voltammetry and the electroactive indicator $Co(phen)_3^{2+}$ was used as label. The results obtained are shown in Figure 2. The peak current of $Co(phen)_3^{3+/2+}$ redox increased greatly and the peak-to-peak separation decreased when the hybridization reaction was conducted. It is considered that the increased peak current is derived from $Co(phen)_3^{3+/2+}$ redox concentrated at the electrode surface through intercalation with formed hybrids. After hybridization, the voltammogram is more reversible than that acquired at a single stranded oligonucleotide modified electrode.

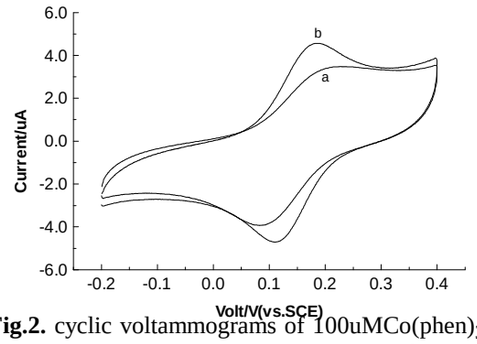

Fig.2. cyclic voltammograms of 100uM$Co(phen)_3^{2+}$ in 5mMTris-50mMNaCl at 100mV/s before(a) and after(b) hybridization

More sensitive electroactive labels which intercalate specifically with dsDNA are under way.

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